LETTERS TO THE EDITOR

Comment on "TCR Activation Eliminates Glutamate Receptor GluR3 from the Cell Surface of Normal Human T Cells, via an Autocrine/Paracrine Granzyme B-Mediated Proteolytic Cleavage"

Being strongly interested in the role of glutamate in the immune system and more specifically on its effects on lymphocyte physiology, we read the paper by Ganor et al. (1) with great interest. We would like to complement the authors for the clear and, for the most part, very well documented data. However, a potentially critical question was raised that we would like to bring to the authors' kind attention and ask for clarification. The question concerns the use of RPMI 1640 as a culture medium in experiments where the effects of 10 nM glutamate were studied. The authors test the ability of 10 nM glutamate to induce T cell adhesion to laminin while cells are bathed in RPMI 1640, a medium with a glutamate concentration of >100 μM (20 mg/L). Could the authors explain how they voided glutamate from the medium and thus were able to evaluate the effects of such a low concentration of glutamate?

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References

1. Ganor, Y., V. I. Teichberg, and M. Levite. 2007. TCR activation eliminates glutamate receptor GluR3 from the cell surface of normal human T cells, via an autocrine/paracrine granzyme B-mediated proteolytic cleavage. *J. Immunol.* 178: 683–692.

Response to Comment on "TCR Activation Eliminates Glutamate Receptor GluR3 from the Cell Surface of Normal Human T Cells, via an Autocrine/Paracrine Granzyme B-Mediated Proteolytic Cleavage"

Glutamate aqueous solutions are notoriously unstable, particularly when glutamate is at concentrations below 1 mM because glutamate forms pyrrolidonecarboxylic acid. In the experiments described in our paper (1), we routinely used an adhesion buffer (i.e., RPMI 1640 supplemented with 0.1% BSA) that was filtered and stored for prolonged periods at 4°C. Such storage resulted in slight pH changes, as deduced by the more pink color of the adhesion buffer. Nevertheless, we used it as such because it never affected the viability of the cells, as was evaluated routinely by trypan blue exclusion or by the experiments themselves.

Thus, we assume that our ability to reliably and repetitively measure the effects of 10 nM glutamate described in our recent paper (1), as well as test a much wider range of 10^{-14}–10^{-4} M glutamate and obtain a consistent dose-response curve for glutamate-induced T cell adhesion, as described in an earlier paper (2), is linked to the aging of the RPMI 1640 and to the disappearance of glutamate that originally was present at the unstable concentration of 136 μM (i.e., 136 micromolar).

The judicious comment of Dr. Poulopoulou (3) now warrants the obvious need to actually measure the glutamate levels in aged RPMI 1640 solutions or try to resuspend the T cells in a glutamate-free medium.

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References

1. Ganor, Y., V. I. Teichberg, and M. Levite. 2007. TCR activation eliminates glutamate receptor GluR3 from the cell surface of normal human T cells, via an autocrine/paracrine granzyme B-mediated proteolytic cleavage. *J. Immunol.* 178: 683–692.
2. Ganor, Y., M. Besser, N. Ben-Zakay, T. Unger, and M. Levite. 2003. Human T cells express a functional ionotropic glutamate receptor GluR3, and glutamate by itself triggers integrin-mediated adhesion to laminin and fibronectin and chemotactic migration. *J. Immunol.* 170: 4362–4372.
3. Poulopoulou, C. 2008. Comment on "TCR activation eliminates glutamate receptor GluR3 from the cell surface of normal human T cells, via an autocrine/paracrine granzyme B-mediated proteolytic cleavage." *J. Immunol.* 180: 2007.

Comment on "Activation-Induced Cytidine Deaminase Expression in Follicular Dendritic Cell Networks and Interfollicular Large B Cells Supports Functionality of Ectopic Lymphoid Neogenesis in Autoimmune Sialoadenitis and MALT Lymphoma in Sjögren's Syndrome"

We read with interest the paper published by Bombardieri et al. (1) in the October 1, 2007 issue of *The Journal of Immunology*.

In line with what we have previously published on chronic allograft rejection (2), the authors report that activation-induced cytidine deaminase (AID) is expressed by B cells of ectopic germinal centers (tertiary lymphoid organs or TLOs)